SUBSECTION MICROBIOLOGY

Nutritional Studies in vitro, with Rous Sarcoma Virus[1]

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An understanding of the relationship between the virus and the infected cell can be approached by kinetic studies of replication of the Rous sarcoma virus in animals (Groupé and Rauscher, 1957; Carr, 1953) or *in vitro*, in cell cultures (Temin and Rubin, 1959).

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Temin and Rubin (1959) studied the kinetics of the infection of chick embryo cells by Rous sarcoma virus and showed that new infectious virus which was produced by these cells was first seen 12 to 14 hours after infection. However, they noted that 24 to 30 hours are required before an average of one focus-forming unit (FFU), specific cytopathology, was released for every originally infected cell. They demonstrated that the amount of free virus increased through 36 hours.

Vigier and Goldé (1959) made a similar kinetic study of Rous sarcoma virus proliferation *in vitro*. They determined that the latent period of Rous sarcoma in virus-infected chick embryo cell monolayers extended from 0 to 24 hours with a steady decrease of virus. The earliest rise in virus titer was between 32 and 40 hours after infection, and could be delayed as long as 72 hours after infection.

The media used by these workers were not clearly chemically defined. Temin and Rubin used a modification of Eagle's medium to which tryptose phosphate broth had been added; the tryptose phosphate broth is not chemically defined. The medium of Vigier and Goldé is less well defined in that it includes calf embryo extract. Both media contained serum, turkey and horse respectively.

This study was designed to grow chick embryo cells *in vitro* in chemically defined medium for the proliferation of Rous sarcoma virus. The defined medium is necessary for studies which are projected to determine the nutrients required by the cell for viral proliferation.

Materials and Methods

Virus: Four to six-week-old Honegger White Leghorn chicks were inoculated in both wing web areas with partially purified Rous sarcoma virus obtained from Dr. W. Ray Bryan, National Cancer Institute, Bethesda, Maryland, for the purpose of producing virus-containing tumors. The virus for tissue culture inoculation was obtained by mincing tumors, allowing them to stand at 4 C for 4 hours and centrifuging at 10,000 x g for 30 minutes. The supernatant fluids were pooled, frozen and stored at -70 C.

Medium: Medium No. 199 (Morgan, Morton and Parker, 1954) was supplemented with 10% pooled calf serum. A commercial preparation (Difco) and medium prepared in this laboratory were used in these studies.

Cell Culture: Nine to eleven-day-old Honegger White Leghorn chick embryos from which feet, wings and head had been removed were trypsinized, washed, the cells counted and resuspended in lactalbumin yeastolate-tryptose phosphate broth medium with added 10% calf serum, were used to initiate primary cell cultures according to the method of Dulbecco and Vogt (1954). After monolayers had formed, these cells were trypsinized, washed and enumerated, then 5 x 10^5 cells in 1 ml of medium 199 were used to establish secondary cultures in tubes. After overnight incubation at 37 C, these cells were washed and infected with Rous sarcoma virus.

Assay of virus: Virus stock suspensions, supernatant fluids, and freeze-thaw preparations of infected cells were assayed for viral content on the chorioallantoic membrane (CAM) of 11-day-old Honegger White Leghorn embryonated eggs according to the method of Rubin (1955, 1957). Titers of virus were expressed as the log of the arithmetic mean of plaque-forming units (PFU).

Results

A growth curve of Rous sarcoma virus in chick embryo cells was performed using a virus input of 4.7 x 10^7 plaque-forming units (PFU) per tube. The titer of virus in the supernatant fluid, free virus (FV), and

infected cells, cell-associated virus (CAV), was determined every two hours over a period of 90 hours. This growth curve can be seen in Figure 1. After a latent period, the virus titer began to increase between the tenth and the twentieth hours, and reached a peak at the thirty-eighth hour. The titer then decreased, which was followed by an increase beginning at the forty-fourth hour and which reached another peak at the sixty-eighth hour. Following this, the titer leveled off through the ninetieth hour. The titer of the CAV was similar but lower.

A second growth curve of the virus in chick embryo cells was performed using a virus input of 6.9×10^3 PFU. It can be seen in Figure 2 that this growth curve of the virus is similar to the growth curve using a higher input of virus (4.7×10^7 PFU), but that not as much virus was produced over a 32-hour period.

A third growth curve of Rous sarcoma virus in chick embryo cells was initiated using a virus input of 6.9×10^3 PFU. The growth medium, after infection, in this experiment contained all the components of the medium 199 except the fat-soluble vitamins. It can be seen in Figure 3 that this growth curve does not differ materially from the preceding growth curve, indicating that apparently an exogenous source of fat-soluble vitamins (A, D, K, E) is not required by the cells to produce the virus over an interval of 32 hours.

Discussion and Summary

The growth curves obtained in this study with more chemically defined medium compare favorably with those of Temin and Rubin (1959) and Vigier and Goldé (1959). A latent period was noted with increase of virus between the tenth and twentieth hours.

The medium is chemically defined except for the serum. The same pool of calf serum was used throughout all the studies, thus, variation was eliminated, facilitating studies of a nutritional nature.

Two growth curves of Rous sarcoma virus, *in vitro,* in chick embryo cell culture, in which a large concentration of virus and a small concentration of virus was used as the inoculum, have been compared. It can be seen that the rate of production of virus over a 32-hour period is not materially different, the only difference being in the relative amount of virus produced.

Two growth curves, in which a low concentration of virus was used as the inoculum, were also compared. The medium for one of the curves was medium No. 199 supplemented with 10% calf serum. The medium used in the other growth curve was medium No. 199, minus the fat-soluble vitamins, and also supplemented with 10% calf serum. It was noted that there was no essential difference between these two curves, thus indicating that an exogenous source of fat-soluble vitamins was not required for the production of the virus.

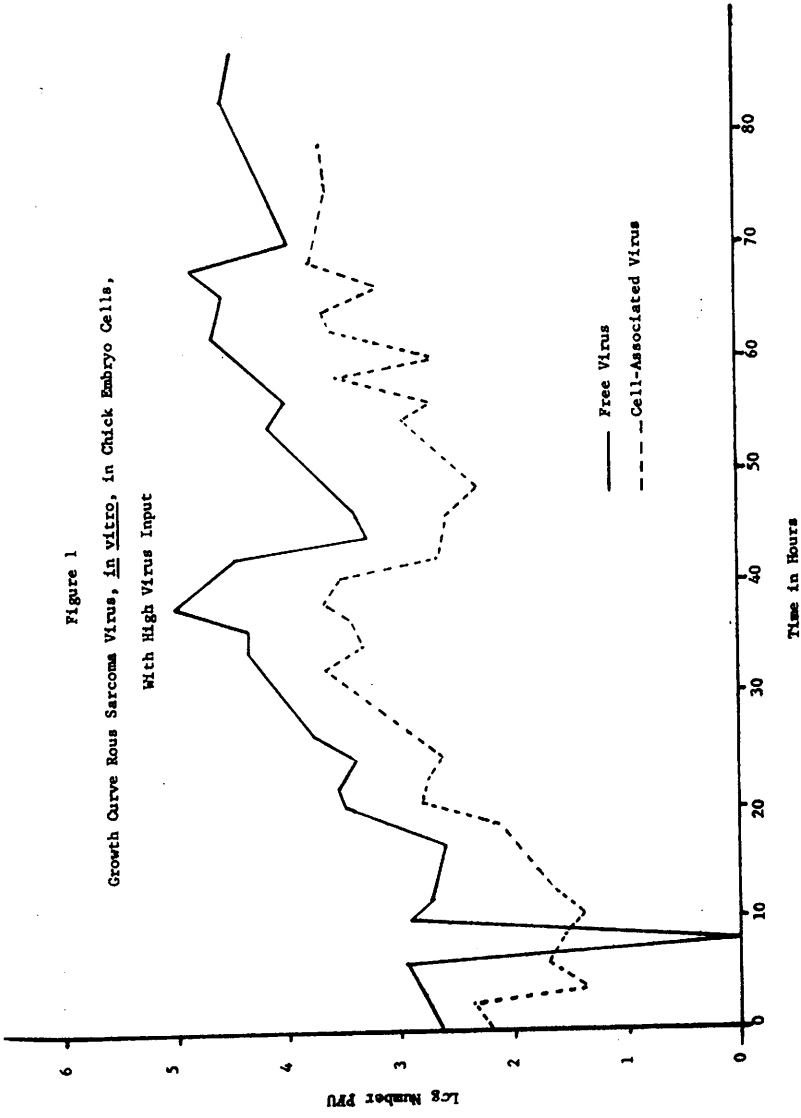


Figure 1

Growth Curve Rous Sarcoma Virus, in vitro, in Chick Embryo Cells, With High Virus Input

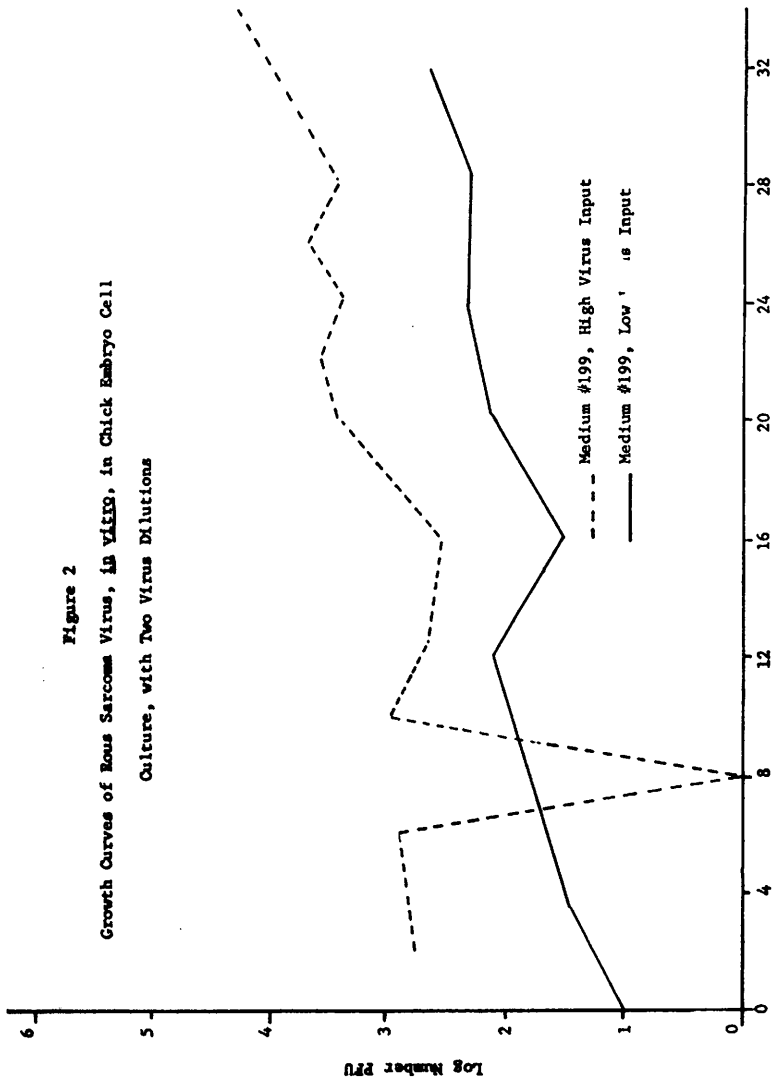
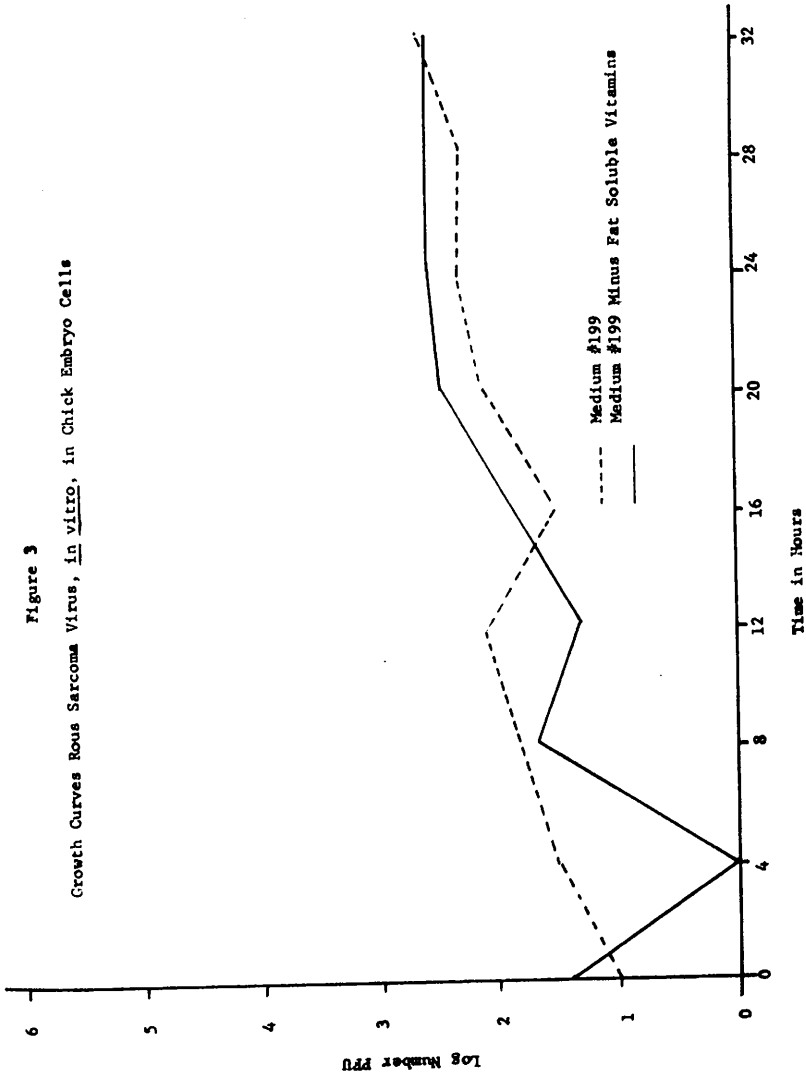


Figure 2

Growth Curves of Rous Sarcoma Virus, in vitro, in Chick Embryo Cell Culture, with Two Virus Dilutions

Figure 3

Growth Curves Rous Sarcoma Virus, in vitro, in Chick Embryo Cells



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